THE ROGER J. WILLIAMS AWARD IN PREVENTIVE NUTRITION

The 1987 recipient of the Roger J. Williams Award in Preventive Nutrition is Richard J. Wurtman of the Massachusetts Institute of Technology. Dr. Wurtman received his undergraduate education at the University of Pennsylvania, and his medical degree from Harvard Medical School. He then joined the staff of the National Institute of Mental Health, where he began his research on brain function. In 1967 he joined the faculty of the Massachusetts Institute of Technology where he continues this research. He has published his work widely in leading scientific journals.

Dr. Wurtman was honored for his outstanding contributions to the understanding of the connection between diet and the normal functioning of the brain. This fundamental research of Dr.Wurtman and his co-workers has important implications for optimizing normal brain function by proper diet. It also has far-reaching implications for our understanding of common abnormalities in eating behavior and for the treatment and possible prevention of disabling diseases of the brain. Such diseases include Alzheimer's disease which tragically robs many of our older citizens of quality life.

The 1987 award was presented on Monday, April 20 at the Texas College of Osteopathic Medicine in Fort Worth, Texas.

The Roger J. Williams Award in Preventive Nutrition is endowed by Mr. and Mrs. E. Bruce Street, Sr., of Graham, Texas. It is presented by the Texas College of Osteopathic Medicine in Fort Worth, Texas. It honors individuals who have made outstanding contributions to the field of preventive medicine. Potential recipients are nominated by individuals familiar with their work, and a screening committee selects three candidates who are considered by a selection panel representing the International Academy of Preventive Medicine, The Clayton Foundation Biochemical Institute of the University of Texas at Austin, and the North Texas State University/Texas College of Osteopathic Medicine Board of Regents. Previous recipients of the award have been William Shive of the University of Texas at Austin, Hector F. DeLuca of the University of Wisconsin, and Robert I. Levy of Columbia University.

CIRCULATING NUTRIENTS AND NEUROTRANSMITTER SYNTHESIS†

Richard J. Wurtman, M.D.*

Meals, snacks and certain purified nutrients can affect the brain and behavior by changing the rate at which neurons synthesize and release neurotransmitters such as serotonin, dopamine and acetylcholine. Consumption of tryptophan or high-carbohydrate meals increases brain levels and release of serotonin; this neurotransmitter has sedative-like effects and decreases appetite for carbohydrate. High-protein meals raise serum tryptophan but paradoxically inhibit its brain uptake, thereby decreasing brain serotonin levels and increasing appetite for carbohydrate. Tyrosine enhances release of catecholamine neurotransmitters and may be useful for depression and Parkinson's disease. Choline or lecithin increase acetylcholine synthesis and release; their consumption can improve tardive dyskinesia, and they are being tested for possible effects in Alzheimer's disease. The unanticipated but well established effects of foods and nutrients on neurotransmitters may lead to improved treatment and prevention of disease, and may lead to future understanding of how some metabolic diseases cause neurological and behavioral disturbances.

Key words: tryptophan, tyrosine, choline, behavior, hyperactivity, aspartame, sugar, carbohydrate, fenfluramine, obesity, affective disorders.

Consumption of a meal or snack, or the administration of particular nutrients that happen to be precursors for monoamine neurotransmitters, can affect the nervous system in an important way: like many drugs, the foods or nutrients can change the rates at which some neurons synthesize and release these neurotransmitters (1,2). The foods or nutrients act by modifying the composition of the plasma — carbohydrates, for example decreasing, and proteins increasing, the plasma concentrations of most of the large neutral amino acids (LNAA) (3,4). And dietary choline or lecithin

†The Roger J. Williams Award Lecture in Preventive Nutrition, April 20, 1987, Texas College of Osteopathic Medicine, Fort Worth, Texas. Some of the studies described in the review were supported by research grants from the National Institutes of Health (NS-21231), the National Institute of Mental Health (MH-28783), the U. S. Air Force (AFOSR 830366), and the National Aeronautics & Space Administration (NAG-2-210), and by a National Institutes of Health grant to the MIT Clinical Research Center (RR00088-24).

*Professor of Neuroendocrine Regulation
Dept. of Brain and Cognitive Sciences and
The Clinical Research Center
[Address for reprints: E25-604]
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

(phosphatidylcholine; PC) increase plasma choline levels (5,6). These changes in plasma composition cause parallel changes in brain amino acid (7,8) and choline (9) levels, which, in turn, affect the rates at which particular neurons convert tryptophan (10), tyrosine (11), and choline (6,12) to serotonin, the catecholamines and acetylcholine, respectively (1,6,13).

This phenomenon — the ability of a meal, depending on its composition, to increase or decrease the production of brain chemicals which mediate communications across synapses — continues to seem very strange: It lacks known counterparts in endocrinology, where eating foods rich in cholesterol does not increase the production of testosterone or estradiol, and eating iodine-containing fish does not enhance thyroxine production in euthyroid individuals. One cannot help but wonder about how it might be advantageous to the body to couple the outputs of certain neurons to food-induced changes in plasma composition, and about how disturbances in this process might underlie aberrant eating behaviors, or disturbances in neuroendocrine secretion, or other abnormalities in brain function. The nutrient-dependence of some neurons also provides physicians with a novel strategy — based on using supplemental nutrients as though they were drugs — for attempting to treat disorders that involve these neurons, and with novel hypotheses for explaining how metabolic diseases that modify plasma composition can also cause neurologic and behavioral disturbances.

Dietary Carbohydrates and Proteins and Brain Serotonin

The initial observation that food consumption can affect neurotransmitter synthesis was made in studies on rats performed in 1971 (10). Animals were allowed to eat a test diet that contained carbohydrates and fat but lacked protein. Soon after the start of the meal, brain levels of the essential (and scarce) amino acid tryptophan were found to have risen, thus increasing the substrate saturation of the enzyme, tryptophan hydroxylase, which controls serotonin synthesis. The resulting increase in brain serotonin levels was also found to have been associated with an increase in serotonin release, as indicated by the concurrent elevation in brain levels of serotonin's chief metabolite, 5-hydroxyindole acetic acid (5-HIAA).

The rise in brain tryptophan levels after the carbohydrate-rich meal was accompanied, in rats but not humans, by a small increase in plasma tryptophan levels. Both of these changes had been unanticipated, since the insulin secretion elicited by dietary carbohydrates was known to *lower* plasma levels of most of the other amino acids (c.f. 3). However, plasma tryptophan's unusual response to insulin was recognized soon thereafter (14) as resulting from another of the amino acid's special properties, namely its propensity to bind loosely to circulating albumin: Insulin causes non-esterified fatty acid (NEFA) molecules to dissociate themselves from albumin and to enter adipocytes. This dissociation increases the protein's capacity to bind circulating tryptophan; hence, whatever reduction insulin causes in "free" plasma tryptophan levels is compensated by a rise in the albumin-bound moiety, yielding, in humans, no net change in total plasma tryptophan levels. (Since this binding is of low

affinity, the albumin-bound tryptophan is almost as able as "free" tryptophan to be taken up into the brain (15)).

Much more difficult to explain were the data subsequently obtained on what happened to brain tryptophan and serotonin levels after rats consumed a meal rich in protein: Although plasma tryptophan levels rose, reflecting the contribution of some of the tryptophan molecules in the protein, brain tryptophan and serotonin levels either failed to rise or, if the meal contained a high proportion of protein, actually fell (7). The explanation for this paradox was found in the kinetic properties of the transport systems that carry tryptophan across the blood-brain barrier (16) and into neurons (17,18): The endothelial cells which line central nervous system capillaries contain eight or more macromolecules which shuttle specific nutrients or their metabolites between the blood and the brain's extracellular space (16). One such macromolecule mediates the transcapillary flux (by facilitated diffusion) of tryptophan and other LNAA; others move choline, basic or acidic amino acids, hexoses, monocarboxylic acids, adenosine, and adenine, and various vitamins. The macromolecule transporting the LNAA — like the one, discussed below, which transports choline — has a poor affinity for its ligands, and thus is not fully saturated at normal plasma LNAA concentrations; hence, an increase in plasma LNAA following consumption of a protein-rich meal can, by suddenly increasing the macromolecule's saturation, rapidly increase LNAA transport into the brain. Moreover, LNAA transport is competitive, so that the ability of circulating tryptophan molecules to enter the brain is increased when plasma levels of the other LNAA fall (as occurs after insulin is secreted (19)), and diminished when the other LNAA rise. Since all dietary proteins are considerably richer in the other LNAA than in tryptophan (which generally comprises only 1.0 - 1.5% of proteins), consumption of a protein-rich meal causes a decline in the "plasma tryptophan ratio" (the ratio of the plasma tryptophan concentration to the summed concentrations of its major circulating competitors for brain uptake: tyrosine; phenylalanine; the branched-chain amino acids leucine, isoleucine, and valine; methionine). This decreases tryptophan's transport into the brain and slows its conversion to serotonin. (Similar competitive mechanisms also mediate the fluxes of tryptophan and other LNAA between the brain's extracellular space and its neurons (17,18); moreover, similar plasma ratios predict brain levels of each of the other LNAA after treatments that modify plasma amino acid patterns (8)).

It seems counter-intuitive that the meal which most effectively raises brain tryptophan levels is the one that lacks tryptophan entirely (that is, one containing carbohydrates but no proteins), while a protein-rich meal, which elevates blood tryptophan concentrations substantially, has the opposite effect on the brain. Plasma tryptophan ratios in normal individuals vary between about 0.065 and 0.160 (3,4), depending largely on the composition of the last meal (or snack) eaten, and the interval that has passed since its ingestion. Such variations are capable, in rats, of causing sizeable differences in brain tryptophan levels (8,13). Subnormal plasma tryptophan

ratios are often noted in obese people, reflecting elevated plasma levels of the branched-chain amino acids (20), perhaps caused by insulin-resistance; these ratios are further reduced if the subjects are put on a high-protein, low-carbohydrate diet (20).†

The fact that giving pure tryptophan can increase brain serotonin synthesis (22), and can thereby affect various serotonin-dependent brain functions (e.g., sleepiness, mood)‡ had been known at least since 1968. What was novel and perhaps surprising about the above experiments was their demonstration that brain tryptophan levels — and serotonin synthesis — *normally* undergo important variations in response, for example, to the decision to eat a carbohydrate-rich vs. a protein-rich breakfast. It remained possible, however, that mechanisms might exist outside the serotonin-releasing neuron itself which kept precursor-induced increases in the transmitter's synthesis from causing parallel changes in the amounts that actually were released into the synapses. Indeed, it was known that if rats were given *very large* doses of tryptophan — sufficient to raise brain tryptophan levels well beyond their normal range — the firing frequencies of their serotonin-releasing raphe neurons decreased markedly (24); this was interpreted as reflecting the operation of a feedback system designed to keep serotonin release within a physiologic range. (Similar decreases in raphe firing had also been observed in animals given drugs, like MAO inhibitors (25) or serotonin-reuptake blockers, which cause persistent increases in intrasynaptic serotonin levels.) However, if rats were given *small* doses of tryptophan — sufficient to raise brain tryptophan levels, but not beyond their normal peaks (26) — or if they consumed a carbohydrate-rich meal, which raised brain tryptophan levels

† How much a meal or snack raises or lowers the plasma tryptophan ratio depends on what else is present in the stomach at the time of its ingestion. Changes in the ratio result from two processes, insulin secretion and the intestinal absorption of amino acids from the dietary protein, both of which depend on the nutrient content of the mixture entering the duodenum. This composition may be very different from that of the meal or snack itself if the stomach was fairly full when the meal or snack was ingested. Note that the ability of a carbohydrate to increase brain serotonin — and thereby modify serotonin-dependent brain functions and behaviors — is independent of its sweetness: A lunch containing 105 g of starch increased the plasma tryptophan ratio as much as one containing even more (123 g) sucrose (21).

‡ Tryptophan is not an approved drug, and physicians who advise its use do so at some legal risk. The same is true for tyrosine, and may or may not be true for PC. I believe that, at present, amino acids should be dispensed for medical uses only to subjects enrolled in approved research protocols. High doses of tryptophan lower brain tyrosine levels through competition for transport at the blood-brain barrier (11,23); hence mildly depressed or insomniac patients who take such doses to enhance brain serotonin synthesis might suffer worse symptoms because of diminished production of brain norepinephrine. There is no compelling evidence that tryptophan-enhanced serotonin synthesis can be increased by administering the amino acid with pyridoxine or any other vitamin (23). Some enhancement can probably be attained, however, by giving it with sufficient dietary carbohydrate to produce an insulin-mediated fall in the plasma levels of the other LNAA (19).

physiologically (27), no decreases in raphe firing occurred.§ Hence, food-induced changes in serotonin synthesis *are* able to increase the amount of serotonin released per firing without slowing the neuron's firing frequencies, and thus are "allowed" to modulate the net output of information from serotoninergic neurons. (This output is, theoretically, the product of three factors: the *number* of serotonin-releasing nerve terminals; the average *frequency* with which the raphe neurons happen to be firing; and the average number of serotonin *molecules released* at each terminal *per firing*.)

Tryptophan, Dietary Carbohydrates, and The Human Brain

The ability of supplemental tryptophan to enhance serotonin turnover within the *human* central nervous system (i.e., to elevate CSF 5-HIAA levels) was first shown in 1970 (31); apparently no neurochemical data are available concerning the human brain's responses to carbohydrate intake. Numerous behavioral and neurological effects (Table 1) have been associated with tryptophan administrations, starting with Smith & Prockop's original observation that it caused drowsiness and euphoria (32). Most of these effects have been reviewed extensively elsewhere (23,34,49,50,51) and are not further discussed here. In experimental animals (13) tryptophan is antihypertensive, and enhances the release of growth hormones; its effects on plasma prolactin levels in man, and on corticosterone levels in the rat, remain uncertain (13).

Only a few well-controlled studies have been published describing behavioral effects of dietary carbohydrates (51). Some of these have involved administering sucrose to hyperactive children whose parents or teachers believed that this carbohydrate exacerbated their behavioral problem. In general, consumption of the sugar tended, if anything, to *reduce* activity (51), similar to its (and tryptophan's) reported effect on normal individuals: a high-carbohydrate lunch increased sleepiness in women, calmness in men, and, in subjects over forty, the tendency to commit errors in a standardized test of performance (52). Apparently, hyperactive children consume larger quantities of sugar than control subjects (53), if they are allowed to do so. This

§ Although normal variations in brain tryptophan levels fail to affect raphe firing, treatments that accelerate raphe firing apparently do modulate the neurons' responses to having additional tryptophan. Supplemental tryptophan causes a much greater increase in serotonin release from active than from quiescent neurons (DeSimoni MG, Sokola A, Fodritto F, Dal Toso G, Aleri S, submitted for publication). This coupling of a serotoninergic neuron's firing frequency to its precursor-responsiveness is similar in some ways to the processes, discussed below, which determine when a catecholaminergic (13,28) or cholinergic (6,29) neuron will respond to changes in tyrosine or choline levels. However, one important difference exists between the precursor-responses of serotoninergic and other monoaminergic neurons: Tryptophan administration (or carbohydrate consumption) invariably causes major increases in brain serotonin *levels*, while tyrosine has little effect on brain dopamine or norepinephrine levels (unless they have been depleted by persistent firing, as occurs in the locus coeruleus of stressed rats) (30). Similarly, choline or PC administration to rats causes only small and inconstant increases in brain acetylcholine (9). Apparently, serotonin synthesis is always coupled to tryptophan levels, whether or not the serotoninergic neuron happens to be active; in contrast, the extent to which catecholamine and acetylcholine synthesis (and release) are affected by supplemental precursors varies with neuronal activity.

TABLE 1

SOME BEHAVIORAL EFFECTS ATTRIBUTED TO TRYPTOPHAN

EFFECT	REFERENCES
Drowsiness, euphoria	32
Decrease time to onset of REM sleep	33
Decrease sleep latency	34
Enhance subjective sleepiness	35,36
Increase sleep time of infants	37
Improve pain tolerance	36,38
Reverse tolerance to analgesics or to analgesic neurosurgery	39,40
Diminish aggression (in schizophrenics)	41
Antidepressant, as adjunct to MAO inhibitors	42
Antidepressant	43
Anti-manic (alone or with lithium)	44,45
Decrease calorie intake	46
Decrease snack carbohydrate intake	47
Promote weight-loss in subjects on high-protein diet	48

These effects are reviewed extensively in references 23,34,49,50 and 51.

could reflect a greater need for energy, or conceivably, an unrecognized attempt at self-medication, similar to that postulated below for patients with the "Seasonal Affective Disorder Syndrome" (SADS) and for other groups of carbohydrate-cravers (54): Perhaps their raphe neurons release "inadequate" quantities of serotonin, causing them to feel dysphoric; consumption of carbohydrates might then ameliorate these feelings, if only temporarily, by augmenting serotonin release. There is evidence that levels of serotonin or 5-HIAA are subnormal in CSF from violent psychiatric patients (23) and in brains of people who have died by suicide (49,50), but apparently there is no information about serotonin levels or turnover in brains of hyperactive children.

Brain Serotonin, Nutrient Choice, and Carbohydrate Craving

If rats are allowed to pick from foods in two pans, presented concurrently, which contain differing proportions of protein and carbohydrate, they choose among the two so as to obtain fairly constant (for each animal) amounts of these macronutrients

(55). However, if prior to "dinner" they receive either a carbohydrate-based "snack" (56) or a drug that facilitates serotoninergic neurotransmission (55), they quickly modify their food choice, selectively diminishing their intake of carbohydrates. These observations support the hypothesis that the responses of serotoninergic neurons to food-induced changes in the plasma amino acid pattern allow these neurons to serve as a "sensor" in the brain's mechanisms governing nutrient choice (54): Perhaps they participate in a feedback loop through which the composition of "breakfast" (that is, its proportions of protein and carbohydrate) can — by increasing or decreasing brain serotonin levels — influence the choice of "lunch" (57).

A similar mechanism may operate in humans. Subjects housed in a research hospital were allowed to choose from six different isocaloric foods (containing varying proportions of protein and carbohydrate, but constant amounts of fat) at each meal, taking as many small portions as they liked; they also had continuous access to a computer-driven vending machine, stocked with mixed carbohydrate-rich and protein-rich isocaloric snacks. The basic parameters of each person's food intake — total number of calories; grams of carbohydrate and protein; number and composition of snacks — tended to vary within only a narrow range, day to day, and to be unaffected by placebo administration (47,58).

To assay the involvement of brain serotonin in maintaining this constancy of nutrient intake, pharmacologic studies were undertaken in individuals in whom the putative feedback mechanism might be inpaired. These were obese people who claimed to suffer from "carbohydrate craving," manifested as their tendency to consume large quantities of carbohydrate-rich snacks, usually at a characteristic time of day or evening (58). Subjects were given d-fenfluramine (Isomerid), a drug which had been found (55) to decrease carbohydrate intake in normal rats, and to cause weight loss in obese people by mechanisms involving the release of brain serotonin. Administration of relatively low doses (15 mg twice daily) caused a major reduction in snack carbohydrate intake (47,58); a smaller reduction in mealtime carbohydrates (58); and no significant changes in mealtime protein (58) nor fat intake. (Too few protein-rich snacks were consumed by the subjects to allow assessment of the drug's effect on this source of calories.) Two other drugs also thought to selectively enhance serotonin-mediated neurotransmission (the antidepressants zymelidine and fluoxetine) likewise cause weight loss. This contrasts with the weight gain (and carbohydrate craving) often associated with less chemically specific antidepressants like amitriptyline. It has not yet been determined whether these drugs also selectively suppress carbohydrate intake in humans.

Severe carbohydrate craving is also characteristic of patients suffering from SADS, a variant of bipolar clinical depression associated with a December or January onset, a higher frequency in populations living far from the equator, and concurrent hypersomnia and weight-gain (59,60). A reciprocal tendency of many obese people to suffer from affective disorders (usually depression) has also been noted (60). Since serotoninergic neurons apparently are involved in the actions of both appetite-

reducing and antidepressant drugs, they might constitute the link between a patient's appetitive and affective symptoms: Some patients with disturbed serotoninergic neurotransmission might seek treatment for obesity, reflecting their overuse of dietary carbohydrates to treat their dysphoria. (The carbohydrates, by increasing intrasynaptic serotonin, would mimic the neurochemical actions of bona fide antidepressant drugs like the MAO inhibitors and tricyclic compounds.) Other patients might complain of depression, and their carbohydrate craving and weight gain would be perceived as secondary problems. A third goup of patients — the bulimics (60) — might seek medical assistance because of their concurrent appetite and psychiatric problems. The participation of serotoninergic drugs in a large number of brain functions besides nutrient-choice regulation might make these functions hostages to eating (seen in the sleepiness that can, for example, follow carbohydrate intake (25)), just as it could cause mood-disturbed individuals to consume large amounts of carbohydrates for reasons related neither to the nutritional value nor to the taste of these foods.

When Will Nutrient Intake Affect Neurotransmission?

On the basis of the tryptophan-serotonin relationship, one can formulate five biochemical processes (1) necessary for any nutrient to affect the synthesis of its neurotransmitter product, and the additional steps required for the nutrient also to affect the release of the neurotransmitter:

1. Plasma levels of the precursor (and of other circulating compounds, like the LNAA for tryptophan that affect its availability to the brain) must be "allowed" to increase after its administration or its consumption in foods. That is, plasma levels of tryptophan or the other LNAA, or of choline, cannot be under tight homeostatic control (like, for example, plasma calcium or osmolarity.) Actually plasma levels of tryptophan, tyrosine, and cholin *do* vary several-fold after consumption of normal foods (3,4,49), and levels of the branched-chain amino acids may vary by as much as five or six-fold (3,4).

2. The brain level of the precursor must be dependent upon its plasma level, i.e., there must not be an absolute blood-brain barrier for circulating tryptophan, tyrosine, or choline. In fact, such absolute barriers do not exist (16); rather, facilitated diffusion mechanisms allow these compounds to enter the brain.

3. The mechanism that couples brain levels of these compounds to plasma composition (that is, to the plasma tryptophan ratio, the plasma tyrosine ratio, or the plasma choline concentration) must be unsaturated, such that a change in plasma amino acid or choline levels can — by enhancing the transport protein's saturation — rapidly accelerate the precursor's entry into the brain. As described above (16), the brain capillary macromolecules that mediate the bidirectional fluxes of LNAA and choline across the blood-brain barrier are, in fact, unsaturated with their ligands.

4. Similarly, the rate-limiting enzyme (within presynaptic nerve terminals) which initiates the conversion of the precursor to its neurotransmitter product must be unsaturated with this substrate. Thus, when presented with more tryptophan, tyrosine, or choline, the enzyme can accelerate synthesis of the neurotransmitter. Indeed, tryptophan hydroxylase (7) and choline acetyltransferase (CAT) (1,9) do have very poor affinities for their substrates tryptophan and choline. As discussed below, tyrosine hydroxylase activity *becomes* tyrosine-limited when neurons containing the enzyme have been activated and the enzyme has been phosphorylated (61,62,63).

5. The activity of the rate-limiting enzyme cannot be subject to local end-product inhibition. That is the products of tryptophan's hydroxylation, 5-hydroxytryptophan and serotonin itself, may not appreciably suppress tryptophan hydroxylase activity, nor may acetylcholine levels within cholinergic nerve terminals affect CAT activity. Tyrosine hydroxylase activity probably is subject to some end-product inhibition when the enzyme protein is in its non-phosphorylated state; however, once the enzyme is phosphorylated, it apparently is freed from this constraint (63).

Available evidence suggests that only some neurotransmitters in the human brain are likely to be subject to such precursor control; principally, the monoamines mentioned above (serotonin; the catecholamines, dopamine, norepinephrine, and epinephrine; and acetylcholine) and, possibly, histidine and glycine. Pharmacologic doses of the amino acid histidine do elevate histamine levels within nerve terminals (64), and the administration of threonine — a substrate for the enzyme that normally forms glycine from serine — can elevate glycine levels within spinal cord neurons (65).

One large family of neurotransmitters, the peptides, almost certainly is *not* subject to precursor control. Brain levels of these compounds have never been shown to change with variations in brain amino acid levels; moreover, there are sound theoretical reasons why it is unlikely that brain peptide synthesis would be thus influenced: The immediate precursor for a brain protein or peptide is not an amino acid, per se (as for some monoamine neurotransmitters) but an amino acid attached to its particular species of tRNA. In brain tissue, the tRNA-charging enzymes characterized to date have very high affinities for their amino acid substrates (66), such that their ability to operate full capacity, *in vivo*, is probably unaffected by amino acid levels (except, possibly, in pathologic states, like phenylketonuria, associated with major disruptions in brain amino acid patterns).

Little is know about the possible precursor control of the non-essential amino acids like glutamate, aspartate, and gamma-aminobutyric acid (GABA) which are probably the most abundant neurotransmitters in the brain, because such relationships are difficult to study. Even though glutamate and aspartate can be formed, at various organs in the body, via many different biochemical pathways, the precise pathways that synthesize these compounds in the terminals of neurons that use them as their neurotransmitters are not well established (67). Although GABA's precursor (glutamate) is well established, brain levels of that amino acid apparently cannot be raised experimentally without sorely disrupting normal brain functions:

The macromolecule that transports acidic amino acids like glutamate and aspartate across the blood-brain barrier is unidirectional, and *secretes* these compounds by an active-transport mechanism from the brain into the blood (16). Hence, administration of even an enormous dose of monosodium glutamate will not affect brain glutamate levels unless it elevates plasma osmolarity to the point of disrupting the blood-brain barrier (68), in which case the experimenter finds himself with a different experiment from the one he intended to perform.

If the monoaminergic neurotransmitters turn out to be the only ones subject to nutritional control, physicians and neuroscientists will still have a number of interesting mechanisms to explore and exploit. These neurotransmitters are critically important in a large number of physiologic mechanisms and pathophysiologic states, and are thought to mediate the actions of many neuropharmacologic agents (69).

In order for an increase in a neurotransmitter's *synthesis* (caused by administering a food or the transmitter's precursor) to affect its *release*, the neuron that releases it must continue to fire at its normal frequency. This may be prevented by receptor-mediated feedback processes which are activated soon after release of the transmitter has been increased (1). One such process involves presynaptic autoreceptors present on many monoaminergic terminals; some transmitter molecules within the synapse interact with the receptors, thereby reducing (by mechanisms not yet fully understood) the number of neurotransmitter molecules released by subsequent firings. Another process involves chains of neurons, including at least one that makes an inhibitory neurotransmitter. The precursor-dependent neurotransmitter now interacts with post-synaptic receptors, ultimately causing the neuron that releases it to receive fewer excitatory (or more inhibitory) impulses, and to fire less frequently. The fact that administration of tyrosine or choline to normal individuals produces few detectable changes in brain function can probably be explained by the operations of these two feedback mechanisms. (Serotonin-releasing neurons, of course, *do not* decrease their firing frequencies when brain tryptophan levels are increased, unless the increase transcends its normal range for these levels (26,27). Hence, brain serotonin synthesis is always responsive to physiologic changes in brain tryptophan levels).

There probably are several situations in which receptor-mediated feedback mechanisms are not activated by increases in transmitter release, thus allowing precursor administration to affect neurotransmission. These might include:

1. *Neurodegenerative disorders*, which diminish the number of neurons (or presynaptic terminals) issuing from a precursor-dependent brain nucleus (e.g., the *substantia nigra* in patients with Parkinson's Disease). The surviving neurons may exhibit increased firing rates (1,49), which makes *them* more sensitive to the precursors, without affecting the precursor-responsiveness of other, intact, neurons that happen to use the same transmitter.
2. *Physiologic circumstances* in which neurons undergo *sustained increases in firing frequency* (e.g., sympatho-adrenal cells in hemorrhagic shock, which respond to supplemental tyrosine by making and releasing more norepinephrine and epinephrins, so long as blood pressure remains low (70).
3. *Peripheral neurons* which, unlike brain, *lack multisynaptic feedback loops* to be activated by increased local neurotransmitter levels. Thus, tyrosine administration enhances catecholamine synthesis in, and release from, peripheral sympathetic neurons (and chromaffin cells) in humans (71). Choline administration (for 2-4 days) also persistently enhances acetylcholine release from splanchnic neurons (causing, among other things, enzyme induction in the post-synaptic adrenomedullary chromaffin cells (72)).
4. Neurons which are components of *positive multisynaptic feedback loops*. In this circumstance, a precursor-induced increase in neurotransmitter release might be expected to cause further activation of the neuron that synthesized the transmitter, and, subsequently, an even greater ability to respond to supplemental precursor.

Tyrosine Effects On Dopamine and Norepinephrine Synthesis

Because tyrosine administration has not been shown to increase brain dopamine or norepinephrine levels, it was assumed until fairly recently that the catecholamine neurotransmitters were not under precursor control, in spite of the fact that (a) plasma tyrosine levels *do* increase several-fold after protein intake (3,4) or tyrosine administration (73); (b) the LNAA transport system does ferry tyrosine, like tryptophan, across the blood-brain barrier, and (c) tyrosine hydroxylase, which catalyzes the rate-limiting step in catecholamine synthesis, is unsaturated *in vivo* (13). It seemed possible that a "pool" of neuronal dopamine or norepinephrine might exist whose synthesis was, indeed, responsive to tyrosine, but that this pool was too small, in relation to total catecholamine levels, to escape detection.

Hence, studies were performed to determine whether catecholamine *synthesis* or *release*, assessed independently of brain catecholamine *levels*, could be affected by changes in brain tyrosine concentrations. Catecholamine synthesis was estimated by following the rate at which dopa, the product of tyrosine's hydroxylation, accumulated in brains of rats treated acutely with a drug that blocks the next enzyme in catecholamine formation (aromatic l-amino acid decarboxylase). Tyrosine administration increased dopa accumulation, while other LNAA decreased both it and brain tyrosine levels (11). Catecholamine *release* was then estimated by measuring brain levels of metabolites of dopamine (homovanillic acid [HVA], dihydroxyphenylacetic acid [DOPAC]) or of norepinephrine (methoxyhydroxyphenylglycol sulfate [MHPH-SO_4]). Administration of even large doses of tyrosine had no consistent effect on these metabolites (13,62). However, if the experimental animals were also given an *additional* treatment designed to *accelerate the firing of* dopaminergic (74) or noradrenergic (75) tracts (e.g., dopamine receptor blockers, cold exposure, partial lesions of dopaminergic tracts, reserpine) the supplemental tyrosine now caused a marked augmentation of catecholamine release. These initial observations formed the basis for the hypothesis that catecholaminergic

neurons *become* tyrosine-sensitive when they are physiologically active, and lose this capacity when they are quiescent.

The biochemical mechanism that couples a neuron's firing frequency to its ability to respond to supplemental tyrosine involves phosphorylation of the tyrosine hydroxylase enzyme protein, a process that occurs when the neurons fire (61,62,63). This phosphorylation, which is short-lived, enhances the enzyme's affinity for its cofactor (tetrahydrobiopterin) and makes it insensitive to end-product inhibition (by norepinephrine and other catechols). These changes allow its net activity to depend on the extent to which it is saturated with tyrosine. An additional mechanism underlying this coupling may be an actual *depletion* of tyrosine within nerve terminals, due to its accelerated conversion to catecholamines. If slices of rat caudate nucleus are superfused with a standard Krebs-Ringers' solution that lacks tyrosine or other amino acids, and are depolarized repeatedly, they are unable to sustain their release of dopamine (Fig. 1) (28); concurrently, their content of tyrosine — but not of other LNAA — declines markedly (28,62). Addition of tyrosine to the superfusion solution enables the tissue to continue releasing dopamine at initial rates, and also protects it against depletion of its tyrosine. The concentrations of tyrosine needed for these effects are proportional to the number of times the neurons are depolarized. (Of course, the intact brain is continuously perfused with tyrosine-containing blood, making it highly unlikely that tyrosine levels fall to a similar extent, even in continuously active brain neurons; however, they might decline somewhat, since tyrosine is poorly soluble in aqueous media, and diffuses relatively slowly.)

The tight coupling of tyrosine-responsiveness to neuronal firing probably explains tyrosine's paradoxical effects on blood pressure (13): The amino acid *elevates* blood pressure (and sympatho-adrenal catecholamine release) in hypotensive animals (70), but *lowers* blood pressure (without effecting sympatho-adrenal catecholamines) in hypertensive animals (76). (It fails to affect blood pressure at all in normotensive animals or humans (13).) Tyrosine's blood-pressure-lowering effect in hypertensive animals probably results from its conversion to norepinephrine in brain stem neurons, which, when active, suppress sympathetic outflow; these neurons presumably are activated in the varieties of hypertension in which tyrosine is effective, participating in the brain's attempts to deal with the hypertension (13). As might be anticipated, tyrosine administration elevates brain levels of MHPG-SO^4 in these animals (76), but has little or no effect in those with normal or low blood pressure.

Supplemental tyrosine may have useful effects in patients with early Parkinson's disease (49) and in depression (given with (50) or without (49) 5-hydroxytryptophan or tryptophan); its utility in treating hypertension or other cardiovascular diseases (e.g., cardiac arrhythmias (77)) awaits evaluation. Tyrosine also may have some value in the prophylaxis or treatment of stress responses. Rats subjected to a standard laboratory stress were found, immediately thereafter, to have depressed brain norepinephrine levels (particularly in the locus coeruleus and hypothalamus), probably reflecting the inability of synthesis to keep up with release; they also showed behavioral

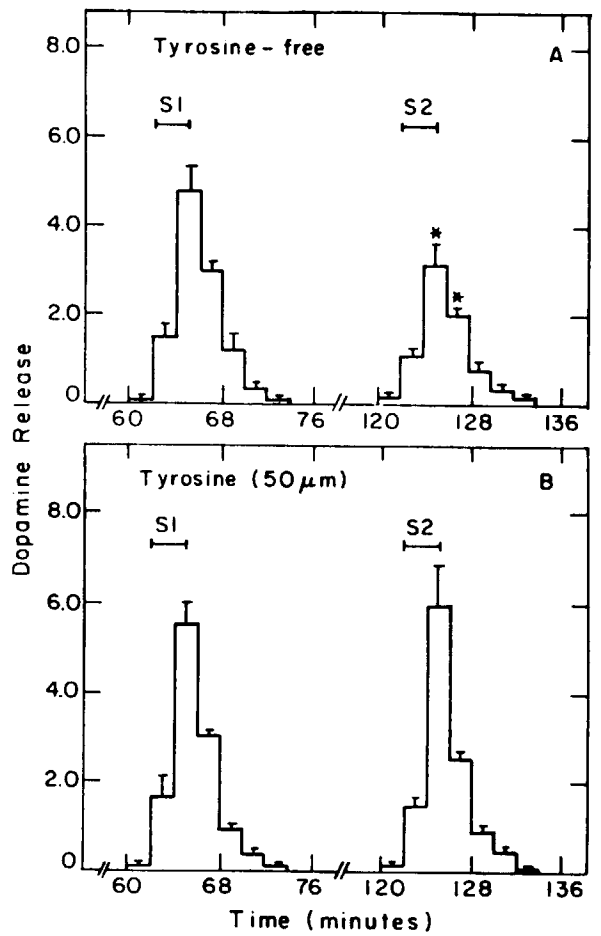

FIGURE 1

Release of endogenous dopamine evoked by electrical stimulation of rat striatal slices, expressed as percentage released of final tissue content. A: tyrosine-free media. B: tyrosine-supplemented media (50 micromolar). S1 and S2 were identical trains of 1800 pulses (60 mA, 2 ms, 20Hz) delivered 60 minutes apart. Superfusate was collected in 2-minute fractions and assayed for dopamine by alumina extraction and high-performance lipid chromatography with electrochemical detection. Data were analyzed by paired t-tests and values shown are mean + SEM for 4 experiments.

* $P < 0.05$ when compared with equivalent fraction from S1. (Reprinted from ref. 28).

abnormalities and elevated plasma corticosterone levels. All these changes, including the adrenocortical response, were suppressed by supplemental oral tyrosine (30,78), but not if the tyrosine was administered with another LNAA (valine) that blocked its brain uptake.

Plasma tyrosine derives from both the tyrosine and the phenylalanine in dietary proteins, since the latter amino acid is hydroxylated to tyrosine in the liver. Phenylalanine itself can apparently serve as a substrate for tyrosine hydroxylase; low concentrations partially sustain dopamine release when tyrosine is lacking in the brain slice preparation discussed above. However, in higher concentrations (200 micromolar), phenylalanine *inhibits* tyrosine hydroxylase activity and suppresses dopamine release (79). This inhibition might become clinically significant in people who consume very large quantities of the dipeptide sweetener aspartame. Unlike dietary proteins, aspartame lacks the other competing LNAA; hence, its consumption can cause major elevations in brain phenylalanine levels, especially if it is consumed with foods containing insulin-releasing carbohydrates (which, as discussed above, lower blood levels of the other LNAA) (80). Dietary proteins, unlike aspartame, fail to elevate (8) and can actually lower brain phenylalanine levels, because proteins contribute much more of the other LNAA than phenylalanine to the circulation. The clinical consequences of an aspartame-induced reduction in brain catecholamine synthesis might be anticipated from the known physiologic and behavioral roles of these neurotransmitters (e.g., maintaining the seizure threshold (81,82)).

Certain widely-used drugs (for example, L-dopa; alpha-methyldopa) are LNAA; like the naturally-occurring LNAA in proteins, their brain levels depend not solely on their own plasma concentrations but on their respective ratios to the other plasma LNAAs. If they are taken with or soon after a high-protein meal, their uptakes into the brain and their therapeutic efficacies will be diminished (83,84); this relationship may explain the "on-off syndrome" in Parkinsonian patients receiving L-dopa.

Choline Or Lecithin: Effects On Acetylcholine Synthesis

The amounts of acetylcholine released by physiologically active cholinergic neurons depend on the concentrations of choline available to them (Fig. 2). In the absence of supplemental free choline, the neurons will continue to release fairly constant quantities of the transmitter (12); however, when choline is made available (in concentrations bracketing the physiologic range), a clear dose relationship is observed between its concentration and acetylcholine release (6,29). (The biochemical mechanism that couples a cholinergic neuron's firing frequency to its choline-responsiveness awaits discovery.) When no free choline is available, the source of the choline used for acetylcholine synthesis is the cells' own membranes (6,12,85). Membranes are very rich in endogenous PC, and this phospholipid serves as a "reservoir" of free choline, much as bone and albumin serve as reservoirs for calcium and essential amino acids. It has been suggested that a prolonged imbalance between the amounts of free choline that are available to a cholinergic neuron and the amounts needed for acetylcholine synthesis, might alter the composition of its membranes to

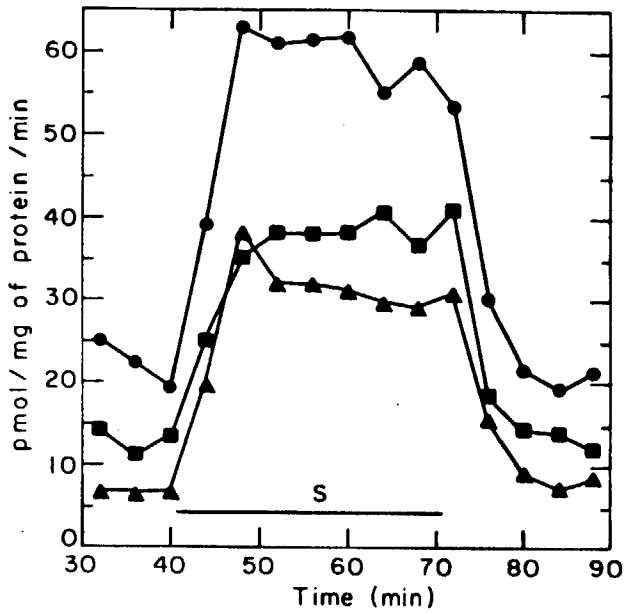

FIGURE 2

Effects of exogenous choline (5 or 20 micromolar) on acetylcholine (ACh) release from rat striatal slices. Tissues were superfused with choline-free physiological solution (▲), 5 micromolar choline (■), or 20 micromolar choline (●). After a 30-minute equilibration, the superfusate was collected at 4-minute intervals. Twelve minutes after the start of the collection period, the slices were electrically stimulated (S) at 15 Hz for 30 minutes (horizontal bar), and then allowed to rest for an 18-minute period. ACh was extracted from the collected fractions and assayed radioenzymatically. The abscissa indicates the time after the start of superfusion; the ordinate represents the rate of ACh release, expressed as pmol/minute, corrected for the milligrams of protein in superfused slices. Each point is the mean of 3 or 4 separate experiments. The addition of either 5 or 20 micromolar choline significantly ($P<0.05$) increased ACh release. (Reprinted from ref. 12).

the point of interfering with its normal functioning, and even the survival of presynaptic terminals ("autocannibalism" (6,86)). In that event, providing the brain with supplemental choline would serve two purposes: It would both enhance acetylcholine release from physiologically active neurons and replenish the choline-containing phospholipids in their membranes.

Neurons can draw on three sources of free choline for acetylcholine synthesis (6): that stored as PC in their own membranes, that formed intra-synaptically from the hydrolysis of acetylcholine (and taken back up into the presynaptic terminal by a high-affinity process estimated to be 30-50 percent efficient in the brain), and that present in the blood stream (and taken into the brain by a specific blood-brain barrier transport system (16)). PC in foods (e.g., liver, eggs) or in nutritional supplements is rapidly hydrolyzed to free choline in the intestinal mucosa (or broken down more slowly, after passage into the lymphatic circulation) (87).‖ Consumption of adequate quantities of PC can lead to several-fold elevations in plasma choline levels, thereby increasing brain choline (9) and the substrate-saturation of CAT.

The phosphatidylcholine consumed in the diet, as well as that formed endogenously in neuronal membranes, is very heterogeneous with respect to fatty-acid composition (6). Some PC's (e.g., those in soy beans and nerve terminals) are relatively rich in polyunsaturated fatty acids; others (e.g., in eggs) are highly saturated. PC's are also heterogeneous with reference to their mode of synthesis (6). Brain neurons produce PC via three distinct biochemical pathways: the sequential methylation of phosphatidylethanolamine [PE], the incorporation of pre-existing free choline via the CDP-choline cycle, or the incorporation of free choline via the base-exchange pathway (in which a choline molecule substitutes for the ethanolamine in PE, or the serine in phosphatidylserine [PS]).

Different types of PC may serve distinct functions. Conceivably, a particular variety of PC (distinguished by its fatty acid composition or its mode of synthesis) is preferentially utilized to provide choline for acetylcholine synthesis; or is preferentially formed during cell division or synaptic remodeling; or is involved in the pathogenesis of particular degenerative diseases afflicting cholinergic neurons (e.g., Alzheimer's disease) (86).

‖ Unfortunately, the term "lecithin" has two different meanings: To physicians and scientists, "lecithin" refers to particular compounds, the phosphatidylecholines (PC's), which may differ in their fatty acid contents but which all contain choline. To the food industry, "lecithin" is simply a mixture of lipids containing at least 95% phosphatides, including, besides PC, compounds (like phosphatidylethanolamine and phosphatidylserine) which lack choline. Almost all "pure lecithin" sold in America contains 20 percent or less authentic PC. Investigators interested in testing the possible therapeutic effects of supplemental PC should restrict their studies to the relatively pure material now available from a few manufacturers, and monitor its effects on plasma choline.

Supplemental choline or PC has been used with success in the treatment of tardive dyskinesia. A recent summary of related publications (88) concluded that choline and the cholinesterase-inhibitor physostigmine were about equally efficacious, and that choline was less toxic. Most patients exhibit some improvement in the frequency of abnormal movements, but only a few show cessation of the movements (49). Choline sources have also been tried in the treatment of Alzheimer's disease (c.f. 89). Most well-controlled studies have treated subjects for relatively short intervals (6-8 weeks or less) and have focused on younger subjects, with little or no success. A single double-blind study administered PC for six months (90). Improvement was noted in about one-third of the subjects. The average age of the responders was 83, and that of non-responders 73, a relationship thought to be compatible with evidence (91) that Alzheimer's disease may be more restricted to cholinergic neurons in subjects who become symptomatic at a later age. It seems important that additional long-term studies now be done on the possible utility of PC in very old Alzheimer's patients. Occasional reports have also described useful effects of choline or PC in treating mania, ataxia, and myasthenic syndromes (49). The general unavailability of purified PC for clinical testing clearly has slowed evaluation of its utility.

Plasma choline levels are markedly elevated during the first postnatal week (92), probably reflecting immaturity of the hepatic choline oxidase enzymes; this provides rapidly growing cells with needed substrate for membrane PC formation. Choline levels were found to be reduced by almost half in athletes completing the Boston Marathon (93).

Conclusions

It appears well established that certain foods and pure nutrients can have important effects on nervous function, effectively modulating the neurotransmissions mediated by serotonin, the catecholamines, and acetylcholine. Brain serotonin synthesis is directly controlled by the proportions of carbohydrate to protein in meals and snacks; these foods increase or decrease brain tryptophan levels, thereby changing the substrate-saturation of tryptophan hydroxylase and the rate of serotonin synthesis. The release of the catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine), from physiologically active brain neurons and sympatho-adrenal cells, is enhanced by tyrosine administration and diminished by the other LNAA, which compete with tyrosine for transport across the blood-brain barrier and neuronal membranes. Acetylcholine synthesis and release can likewise be amplified in physiologically active neurons by consumption of PC-rich foods.

The ability of serotoninergic neurons to have their output coupled to dietary macronutrients allows them to function as "sensors" of peripheral metabolism, and to serve an important role in the control of appetite. However, it also makes the numerous other functions mediated by these neurons (e.g., sleepiness, mood) vulnerable to food intake, and may explain why some obese or depressed people overconsume dietary carbohydrates, perhaps using these foods as though they were antidepressant drugs (which also tend to increase intrasynaptic serotonin). The robust and selective responses of catecholaminergic and cholinergic neurons to supplemental

tyrosine and choline raise the possibility that these compounds (or typtophan, for serotoninergic neurons) may become useful as a new type of "drug" for treating diseases or conditions in which adequate quantities of the transmitter would otherwise be unavailable. If these nutrients turn out to have useful therapeutic properties, their utility will no doubt be enhanced by their familiarity to the body (which has no difficulty metabolizing large amounts of them overnight, without a trace); by their specificity in requiring the collaboration of individual neurons (which must both convert them to their neurotransmitter product and keep firing) to be effective; and by the ease with which their brain levels can be estimated from measurements of plasma composition. However, these advantages are counter-balanced by some obvious disadvantages in comparison with "real" drugs: They are much less potent (and, indeed, lack intrinsic potency at synapses), and they seem to generate less enthusiasm for development than "new" chemicals invented in pharmaceutical companies' own laboratories.

References

1. Wurtman RJ, Hefti F, Melamed E. 1980. Precursor control of neurotransmitter synthesis. Pharmacol Reviews 32:315-335.
2. Wurtman RJ. Nutrients that modify brain function. 1982. Sci Am 246:42-51.
3. Fernstrom JD, Wurtman RJ, Hammarstrom-Wiklund B, Rand WM, Munro HN, Davidson CS. 1979. Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino acids: effect of dietary protein intake. Am J Clin Nutr 32:1912-1922.
4. Maher TJ, Glaeser BS, Wurtman RJ. 1984. Diurnal variations in plasma concentrations of basic and neutral amino acids in red cell concentrations of aspartate and glutamate: Effects of dietary protein intake. Am J Clin Nutr 39:722-729.
5. Wurtman RJ, Hirsch MJ, Growdon J. 1977. Lecithin consumption raises serum-free choline levels. Lancet 2:68-69.
6. Blusztajn JK, Wurtman RJ. 1983. Choline and cholinergic neurons. Science 221:614-620.
7. Fernstrom JD, Wurtman RJ. 1972. Brain serotonin content: Physiological regulation by plasma neutral amino acids. Science 178:414-416.
8. Fernstrom JD, Faller DV. 1978. Neutral amino acids in the brain: Changes in response to food ingestion. J Neurochem 30:1531-1538.
9. Haubrich DR, Gerber NH, Pflueger AB. 1979. Choline availability and the synthesis of acetylcholine. In: Barbeau A, Growdon JH, Wurtman RJ. eds. Choline and lecithin in brain disorders. New York: Raven Press, 57-71.
10. Fernstrom JD, Wurtman RJ. 1971. Brain serotonin content: Physiological dependence on plasma tryptophan levels. Science 173:149-152.
11. Wurtman RJ, Larin F, Mostafapour S, Fernstrom JD. 1974. Brain catechol synthesis: Control of brain tyrosine concentration. Science 185:183-184.
12. Maire JC, Wurtman RJ. 1985. Effects of electrical stimulation and choline availability on release and contents of acetylcholine and choline in superfused slices from rat striatum. J Physiol 80:189-195.
13. Sved AF. 1983. Precursor control of the function of monoaminergic neurons. In: Wurtman RJ, Wurtman JD. eds. Nutrition and the brain, Vol. 6. New York: Raven Press, 223-275.
14. Madras BK, Cohen EL, Messing R, Munro HN, Wurtman RJ. 1974. Relevance of serum-free tryptophan to tissue tryptophan concentrations. Metab 23:1107-1116.

15. Yuwiler A, Oldendorf WH, Geller E, Braun L. 1977. Effect of albumin binding and amino acid concentration on tryptophan uptake into brain. J Neurochem 28:1015-1023.
16. Pardridge WM. 1977. Regulation of amino acid availability to the brain. In: Wurtman RJ, Wurtman JJ. eds. Nutrition and the brain, Vol. 7. New York: Raven Press, 141-204.
17. Lajtha A. 1974. Amino acid transport in the brain *in vivo* and *in vitro*. In: Wolstenholme GEW, Fitzsimmons DW. eds. London: Elsevier, Ciba Foundation Symposium 22, 25-41.
18. Moore MC, Wurtman RJ. 1981. Characteristics of synaptosomal tyrosine uptake: Relation to brain regions and to presence of other amino acids. Life Sci 28:65-75.
19. Martin-Du-Pan R, Mauron C, Glaeser B, Wurtman RJ. 1982. Effect of increasing oral glucose doses on plasma neutral amino acid levels. Metab 31:937-943.
20. Heraief E, Burckhardt P, Mauron, Wurtman J, Wurtman RJ. 1983. The treatment of obesity by carbohydrate deprivation suppresses plasma tryptophan and its ratio to other large amino acids. J Neural Transm 57:187-195.
21. Lieberman HR, Caballero B, Finer N. 1986. The composition of lunch determines afternoon plasma tryptophan ratios in humans. J Neural Transm 65:211-217.
22. Ashcroft GW, Eccleston D, Crawford TBB. 1965. 5-hydroxyindole metabolism in rat brain: A study of intermediate metabolism using the techniques of tryptophan loading. J Neurochem 12:483-492.
23. Young S. 1985. The clinical psychopharmacology of tryptophan. In: Wurtman RJ, Wurtman JJ. eds. Nutrition and the brain, Vol. 7. New York: Raven Press, 49-88.
24. Gallager DW, Aghajanian GK, 1976. Inhibition of firing of raphe neurons by tryptophan and 5-hydroxytryptopha: Blockage by inhibiting serotonin synthesis with RO4-4602. Neuropharmacology, 15:149-158.
25. Aghajanian GK, Graham AW, Sheard MH. 1970. Serotonin-containing neurons in brain: Depression of firing by monoamine oxidase inhibitors. Science 169:1100-1102.
26. Bramwell GJ. 1974. Factors affecting the activity of 5-HT-containing neurons. Brain Res 79:515-519.
27. Trulson ME. 1985. Dietary tryptophan does not alter the function of brain serotonin neurons. Life Sci 37:1067-1072.
28. Milner JD, Wurtman RJ. 1985. Tyrosine availability determines stimulus-evoked dopamine release from rat striatal slices. Neurosci Lett 59:215-220.
29. Bierkamper GG, Goldberg AM. 1980. Release of acetylcholine from the vascular perfused rat phrenic nerve-hemidiaphragm. Brain Res 202:234-237.
30. Lehnert H, Reinstein DK, Strowbridge BW, Wurtman RJ. 1984. Neurochemical and behavioral consequences of acute, uncontrollable stress: Effect of dietary tyrosine. Brain Res 303:215-223.
31. Eccleston D, Ashcroft GW, Crawford TBB, Stanton JB, Wood D, McTurk PH. 1970. Effect of tryptophan administration on 5-HIAA in cerebrospinal fluid in man. J Neurol Neurosurg Psychiatry 33:269-272.
32. Smith B, Prockup DJ. 1962. Central-nervous-system effects of ingestion of L-tryptophan by normal subjects. N Engl J Med 267:1338-1341.
33. Oswald I, Ashcroft GW, Berger RJ, Eccleston D, Evans JI, Thacore VR. 1966. Some experiments in the chemistry of normal sleep. Br J Psychiatry 112:391-399.
34. Hartmann E, Greenwald D. 1984. Tryptophan and human sleep: An analysis of 43 studies. In: Schlossberg W, Kochen B, Linzen B, Steinhart E. eds. Progress in tryptophan and serotonin research. Berlin: Walter de Gruyter, 297-304.
35. Hartmann E, Spinweber CL, Ware C. 1976. L-tryptophan, L-leucine, and placebo: Effects on subjective alertness. Sleep Res 5:57.
36. Lieberman HR, Corkin S, Spring BJ, Growdon JH, Wurtman RJ. 1983. Mood, performance, and pain sensitivity: changes induced by food constituents. J Psychiatric Res 17:135-145.
37. Yogman MW, Zeisel SH. 1983. Diet and sleep patterns in newborn infants. N Eng J Med 309:1147-1149.

38. Seltzer S, Stoch R, Marcus R, Jackson E. 1982. Alteration of human pain thresholds by nutritional manipulation and l-tryptophan supplementation. Pain 13:385-393.

39. Hosobuchi Y, Lamb S, Baskin D. 1980. Tryptophan loading may reverse tolerance to opiate analgesics in humans: A preliminary report. Pain 9:161-169.

40. King RB. 1980. Pain and tryptophan. J Neurosurg 53:44-52.

41. Morand C, Young SN, Ervin FR. 1983. Clinical Response of aggressive schizophrenics to oral tryptophan. Biol Psychiatry 18:575-578.

42. Coppen A, Shaw DM, Farrel JP. 1963. Potentiation of the anti-depressant effect of a monoamine-oxidase inhibitor by tryptophan. Lancet i:79-81.

43. Moller SE, Kirk L, Honore P. 1980. Relationship between plasma ratio of tryptophan to competing amino acids and the response to L-tryptophan treatment in endogenously depressed patients. J Affective Disord 2:47-49.

44. Brewerton TD, Reus VI. 1983. Lithium carbonate and L-tryptophan in the treatment of bipolar and schizoaffective disorders. Am J Psychiatry 140:757-760.

45. Murphy DL, Baker M, Goodwin FK, MillerL, Kotin J, Bunney WE. 1974. L-tryptophan in affective disorders: Indoleamine changes and differential clinical effects. Psychopharmacology 34:11-20.

46. Hrboticky N, Leiter LA, Anderson GH. 1985. Effects of L-tryptophan on short-term food intake, subjective hunger and mood in healthy lean men. Nutr Res 5:595-607.

47. Wurtman JJ, Wurtman RJ, Growdon JH, Lipscomb Henry P, Zeisel SA. 1981. Carbohydrate craving in obese people: Suppression by treatments affecting serotoninergic transmission. Int J Eating Disord 1:2-15.

48. Heraief E, Burckhardt P, Wurtman JJ, Wurtman RJ. 1985. Tryptophan administration enhances weight loss by some moderately-obese patients on a protein-sparing modified fast (PSMF) diet. Int Eating Disord 4:281-292.

49. Growdon JH. 1979. Neurotransmitter precursors in the diet: their use in the treatment of brain disease. In: Wurtman RJ, Wurtman JJ. eds. Nutrition and the brain, Vol. 3, New York: Raven Press, 117-181.

50. van Praag HM, Lemus C. 1985. Monoamine precursors in the treastment of psychiatric disorders. In: Wurtman RJ, Wurtman JJ. eds. Nutrition and the brain, Vol 7, New York: Raven Press, 89-138.

51. Spring B. 1985. Effects of foods and nutrients on the behavior of normal individuals. In: Wurtman RJ, Wurtman JJ. eds. Nutrition and the brain, Vol. 7, New York: Raven Press, 1-47.

52. Spring B, Maller O, Wurtman JJ, Digman L, Cozolino L. 1983. Effects of protein and carbohydrate meals on mood and performance: Interactions with sex and age. J Psychiatr Res 17:155-167.

53. Prinz RJ, Roberts WA, Hartman E. 1980. Dietary correlates of hyperactive behavior in children. J Consult Clin Psychol 48:760-769.

54. Wurtman RJ. 1983. Behavioural effects of nutrients. Lancet 1:145-147.

55. Wurtman JJ, Wurtman RJ. 1979. Drugs that enhance central serotoninergic transmission diminish elective carbohydrate consumption by rats. Life Sci 24:895-904.

56. Wurtman JJ, Moses PL, Wurtman RJ. 1983. Prior carbohydrate consumption affects the amount of carbohydrate that rats choose to eat. J Nutr 113:70-78.

57. Theall CL, Wurtman JJ, Wurtman RJ. 1984. Self-selection and regulation of protein-carbohydrate that rats choose to eat. J Nutr 114:711-718.

58. Wurtman JJ, Wurtman RJ, Mark S, Tsay R, Wilbert W, Growdon J. 1985. D-fenfluramine selectively suppresses carbohydrate snacking by obese subjects. Int Eating Disord 4:89-99.

59. Rosenthal NE, Sack DA, Carpenter CJ, Parry BL, Mendelson WB, Wehr TA. 1985. Antidepressant effects of light in seasonal affective disorder. Am J Psychiatry 142(2):163-170.,

60. Rosenthal NE, Hefferman MM. 1985. Bulimia, carbohydrate craving, and depression: A central connection: In: Wurtman RJ, Wurtman JJ. eds. Nutrition and the brain, Vol. 7, New York: Raven Press, 139-166.

61. El Mestikaway S, Glowinski J, Hamon M. 1983. Tyrosine hydroxylase activation in depolarized dopaminergic terminals: involvement of calcium. Nature, 302:830-832.

62. Milner JD, Wurtman RJ. 1986. Catecholamine synthesis: Physiological coupling to precursor supply. Biochem Pharmacol 35:875-881.

63. Lovenberg W, Ames MM, Lerner P. 1978. Mechanism of short-term regulation of tyrosine hydroxylase. In: Lipton MA, DiMascio A, Killam KF. Psychopharmacology: a generation of progress. New York: Raven Press, 247-259.

64. Schwarz JC, Lampart C, Rose C. 1972. Histamine formation in rat brain *in vivo*: Effects of histidine loads. J Neurochem 19:801-810.

65. Maher TJ, Wurtman RJ. 1980. L-threonine administration increases glycine concentrations in the rat central vervous system. Life Sci 26:1283-1286.

66. Barra HS, Unates LE, Saavedra MS, Capputo R. 1972. Capacities of binding amino acids by tRNAs from rat brain and their changes during development. J Neurochem 19:2287-2297.

67. Wroblewski JT, Blaker WD, Meed JL. 1985. Ornithine as a precursor of neurotransmitter glutamate: Effect of canaline on ornithine aminotransferase activity and glutamate content in the septum of rat brain. Brain Res 329:161-168.

68. McCall A, Glaeser BS, Millington W, Wurtman RJ. 1979. Monosodium glutamate neurotoxicty, hyperosmolarity, and blood-brain barrier dysfunction. Neurobehav Toxicol 1:279-283.

69. Neurotransmitters and CNS Disease: A Lancet Review. The Lancet, 1982; October 23 - December 18.

70. Conlay LA, Maher TJ, Wurtman RJ. 1981. Tyrosine increases blood pressure in hemorrhagic shock. Science, 212:559-560.

71. Agharanya J, Alonso R, Wurtman RJ. 1981. Changes in catecholamine excretion following tyrosine ingestion in normally-fed human subjects: Am J Clin Nutr 34:82-87.

72. Ulus IH, Hirsch MC, Wurtman RJ. 1977. Transsynaptic induction of adrenal tyrosine hydroxylase activity by choline: Evidence that choline administration increases cholinergic transmission. Natl Acad Sci 74:798-800.

73. Melamed E, Glaeser B, Growdon JH, Wurtman RJ. 1980. Plasma tyrosine in normal humans: Effects of oral tyrosine and protein-containing meals. J Neural Transm 47:299-306.

74. Scally MC, Ulus I, Wurtman RJ. 1977. Brain tyrosine level controls striatal dopamine synthesis in haloperidol-treated rats. J Neural Transm 41:1-6.

75. Gibson CJ, Wurtman RJ. 1978. Physiological control of brain norepinephrine synthesis by brain tyrosine concentration. Life Sci 22:1399-1406.

76. Sved AF, Fernstrom JD, Wurtman RJ. 1979. Tyrosine administration reduces blood pressure and enhances brain norepinephrine release in spontaneously-hypertensive rats. Proc Natl Acad Sci USA 76:3511-3514.

77. Scott NA, DeSilva RA, Lown B, Wurtman RJ. 1981. Tyrosine administration decreases vulnerability to ventricular fibrillation in the normal canine heart. Science 211:727-729.

78. Reinstein DK, Lehnert H, Wurtman RJ. 1985. Dietary tyrosine suppresses the rise in plasma corticosterone following acute stress in rats. Life Sci 37:2157-2163.

79. Milner JD, Irie K, Wurtman RJ. 1986. Phenylalanine inhibition and enhancement of endogenous dopamine release from rat striatal slices. J Neurochem 47:1444-1448.

80. Yokogoshi H, Roberts CH, Caballero B, Wurtman RJ. 1984.Effects of aspartame and glucose administration on brain plasma levels of large neutral amino acids and brain 5-hydroxyindoles. Am J Clin Nutr 40:1-7.

81. Jobe PC, Ko KH, Daily JW. 1984. Abnormalities in norepinephrine turnover rate in the central nervous system of the genetically epilepsy-prone rat. Brain Res 290:357-360.

82. Wurtman RJ. 1985. Aspartame: possible effect on seizure susceptibility. The Lancet p. 1060.

83. Markovitz D, Fernstrom JD. 1977. Diet and uptake of adomet by the brain: Competition with natural large neutral amino acids. Science 197:1014.

84. Nutt JG, Woodward WR, Hammarstad JP, Carter JH. 1984. The "on-off" phenomenon in Parkinson's disease: Relation to levodopa absorption and transport. N Eng J Med 310:483-488.

85. Parducz A, Diss Z, Joo P. 1976. Changes in the phosphatidylecholine content and the number of synaptic vesicles in relation to the neurohumoral transmission in sympathetic ganglia. Experientia 32:1520-1521.

86. Wurtman RJ. 1985. Alzheimer's disease. Sci Am 252:62-75.

87. Houtsmuller UMT. 1979. Metabolic Fate of Dietary Lecithin. In: Barbeau A, Growdon JH, Wurtman RJ. eds. Choline and lecithin in brain disorders. New York: Raven Press, 83-94.

88. Nasrallah HA, Dunner FL, Smith RE. 1984. McCalley-Whitters M, Sherman AD. Variable clinical response to choline in tardive dyskinesia. Psychol Med 14:697-700.

89. Corkin S, David KL, Growdon JH, Usdin E, Wurtman RJ. eds. 1982. Alzheimer's Disease: a report on progress in research. New York: Raven Press, pp.525.

90. Little A, Levy R, Chaqui-Kidd P, Hand D. 1985. A double-blind placebo controlled trial of high-dose lecithin in Alzheimer's disease. J Neurol Neurosurg Psychiatry 48:736-742.

91. Rossor MN, Garret RJ, Johnson AL, Mountjoy CQ, Roth M, Iversen LL. 1982. A post-mortem study of the cholinergic and GABA systems in senile dementia. Brain 105:313-318.

92. Zeisel SH, Epstein JHF, Wurtman RJ. 1980. Elevated choline concentrations in neonatal plasma. Life Sci 26:1827-1831.

93. Conlay L, Wurtman RJ, Blusztajn JK, Lopez G, Coviella I, Maher TJ, Evoniuk GE. 1986. Marathon running decreases plasma choline concentration. N Eng Med 315:892.

DIET, ENDOTHELIAL PERMEABILITY, AND ATHEROSCLEROSIS

Bernhard Hennig, Ph.D.*

Cardiovascular disease remains the leading cause of death in the United States. Several lines of evidence suggest that part of the etiology of atherosclerosis involves damage to the vascular endothelium. This reduces its effectiveness as a selective permeability barrier to plasma components. Vascular endothelial cells normally are the only cells in the arterial wall exposed to high concentrations of lipoproteins which are rich in triglycerides and cholesterol. It has been suggested that excessive amounts of fatty acid anions, liberated during lipoprotein triglyceride hydrolysis, may cause localized endothelial injury. This may facilitate the penetration of cholesterol-rich remnant lipoproteins derived from chylomicrons or VLDL into the arterial wall, leading to lipid accumulation within the intima and ultimate plaque formation. To reduce endothelial injury and the accumulation of cholesterol in the arterial wall, dietary treatment should include both caloric balance and a decrease in total lipid intake. Key words: Diet, atherosclerosis, plasma lipids, endothelial damage

Introduction

Atherosclerosis remains the leading cause of death in the United States. In this disease cholesterol accumulates in the wall of arteries and forms bulky plaques that inhibit the flow of blood. Eventually a clot may form, which in turn obstructs arterial blood flow and leads to a myocardial infarction or stroke. Cholesterol-rich blood components include low density lipoproteins (LDL) and chylomicron remnants, which are derived from very low density lipoproteins (VLDL) and chylomicrons, respectively. Chylomicrons carry diet-derived lipids, whereas VLDL carry lipids synthesized in the liver from excess carbohydrates. LDL is the major carrier of cholesterol in the blood (22), and much evidence suggests a positive correlation between plasma LDL levels and the development of atherosclerosis (4,24). Free fatty acids generated during triglyceride hydrolysis have been hypothesized to be injurious to the endothelium (30). This may facilitate the uptake of cholesterol-rich lipoproteins into the blood vessel wall.

Without ignoring other independent risk factors associated with cardiovascular disease such as smoking, diabetes mellitus, and hypertension, medical professionals and nutritionists often have to advise the public concerning diets which reduce the risk for cardiovascular disease. As I will describe, this advice should include caloric restriction and a decrease in total lipid intake to maintain plasma triglyceride-rich lipoprotein levels (chylomicrons and VLDL) at their minimum.

*Assistant Professor
Department of Nutrition and Food Science
University of Kentucky
Lexington, Kentucky 40506-0054